

Appl. No. 10/633,742
Docket No. 9045M
Amdt. dated 31 October 2006
Reply to Office Action mailed on 1 May 2006
Customer No. 27752

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Please delete the paragraph beginning at page 23, line 1, and insert the following paragraph therefore:

"Methods: Expression and purification of recombinant HPTPbeta catalytic domain [SEQ ID NO: 16] is accomplished by subcloning the intracellular domain of HPTPbeta (cDNA clone starting at the codon for amino acid 1662 through the stop codon) into the pGEX-4T1 vector (Pharmacia) and expressed as a GST-fusion protein in *E.coli* BL21-RIL cells (Stratagene). Induced cells are thawed on ice and resuspended in 20mM Tris-HCl, pH=8.0, 1% Triton x-100, 2mM DTT and EDTA-free protease inhibitor cocktail. 10mg/ml lysozyme is added and cells are lysed by sonication. The cell debris is pelleted using the Beckman J2-MI at 17,000 rpm. The supernatant is added to prepared glutathione sepharose 4B resin batch method. After binding, the column is poured and packed with the resin/protein mixture and then washed. The column is unpacked for cleavage of GST using thrombin (50 units thrombin/1mL resin). Mixture is rotated O/N at room temperature. The column is re-packed for washing cleaved material from resin. Cleaved material is collected and then loaded onto Q sepharose FF column. The column is washed and protein eluted with 0-1M NaCl gradient. A coomassie blue stained SDS-PAGE gel identified fractions containing recombinant material and pooled fractions and dialyzed against 20mM Tris-HCl, pH 8.0, 10mM BME, 2mM DTT."

Please delete the paragraph beginning at page 23, line 21, and insert the following paragraph therefore:

"To prepare recombinant GST fusion receptor tyrosine kinase domains for kinase domain dephosphorylation assays, intracellular kinase domain cDNAs of the VEGFR2 [SEQ ID NO: 11] and Tie-2 [SEQ ID NO: 13] receptors are generated by PCR starting at the codon for the 1st amino acid inside the transmembrane domain, a lysine in both receptors, using forward primers having

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an "in-frame" Sal I site and reverse primer containing a Not I site for both receptors. The pLNCX2 plasmids below are used as templates. PCR fragments are subcloned into the GST fusion vector pACGHLT-A (Pharmingen/BD Biosciences) and sequenced through on both strands. Confirmed clones are transfected into sf9 insect cells using the BaculoGold™ system from Pharmingen. Resulting baculovirus stocks are amplified and final protein production is rendered in infected sf9 cells on 150mm dishes. Infected sf9 cells were lysed in 2mL of Triton Lysis Buffer (TLB, 20mM Tris-HCl, pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 1mM Sodium Orthovanadate, 1mM Sodium Fluoride, 1 mM PMSF, 1 ug/ml leupeptin, 1 ug/ml pepstatin), scraped, and frozen at -80°C until used."

Please delete the paragraph beginning at page 25, line 7, and insert the following paragraph therefore:

"For phosphatase activity in human embryonic kidney cells, HEK293H (Gibco) are grown in suspension in 293SFM media (Gibco) and transfected on 100 mm dishes using Lipofectamine LIPOFECTAMINE™ 2000 (Invitrogen), a proprietary formulation for the transfection of nucleic acids (DNA and RNA) into eukaryotic cells. After 24 hours cells are resuspended transferred back to shaker flasks for an additional 24 hours. Phosphatase activity is measured using DiFMUP in 96 well plates as above with 100,000 cells/well. For inhibition of tyrosine phosphatases, the cells are pre-incubated with Bis-maloalto-organovanadium (BMOV) for 15 minutes prior to the addition of DIFMUP. HPTPbeta protein expression is confirmed after each experiment in parallel plates by western blotting."

Please delete the paragraph beginning at page 25, line 14, and insert the following paragraph therefore:

"To establish stable cell lines expressing endothelial cell receptor tyrosine kinases, HEK293 cells are transfected with mammalian expression plasmids

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(pLNCX2, Clontech) containing either the full length human VEGF receptor type 2 (VEGFR2) [SEQ ID NO: 6] cDNA or full length human Tie-2 receptor [SEQ ID NO: 8] cDNA using ~~LipofectAMINE-Plus~~ LIPOFECTAMINE™ PLUS (Invitrogen), a reagent used in conjunction with transfection reagents to enhance transfection in adherent cell lines, on 100mm dishes. Forty-eight hours post transfection the cells are selected in growth medium (DMEM, 10% fetal bovine serum) supplemented with 1.4 mg/mL ~~Geneticin™~~ GENETICIN™ (Gibco), an analog of neomycin sulfate which interferes with the function of 80S ribosomes and protein synthesis in eukaryotic cells (the concentration of ~~Geneticin™~~ GENETICIN™ was determined by kill curve analysis of wild type HEK293 cells). After selection, isolated colonies are chosen using cloning cyclinders and the clonal cell lines propagated in complete selection media. Clonal cell lines were screened for the presence of the receptors by western blot as well as by receptor activation assay. The Tie-2 stable cells are designated T2-3 and the VEGFR2 stable cells designated R2-6.”

Please delete the paragraph beginning at page 27, line 30, and insert the following paragraph therefore:

“**Methods:** Antisense (AS) oligonucleotides capable of selective inhibition of HPTPbeta expression are supplied by Sequitur, Inc. (Natick, MA). Design and screening of the HPTPbeta antisense oligonucleotides (AS) is accomplished as follows: ten phosphorothioate-containing DNA oligonucleotides with perfect 25 nucleotide complementarity to selected regions of the HPTPbeta coding sequence were synthesized by standard phosphoramidite chemistry and purified with trityl- on using reverse-phase columns followed by de-salting on a size exclusion matrix. Chemistry-matched control oligomers, lacking significant sequence homology to known or predicted human genes, are made in parallel. Antisense and control oligonucleotides are transfected into Human Microvascular Endothelial Cells with ~~Lipofectamine~~ LIPOFECTAMINE™ 2000 using conditions recommended by the

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manufacturer (Invitrogen, Inc.). Uptake efficiency is monitored in live cells using a fluorescently labeled oligonucleotide as described by the manufacturer (Sequitur, Inc.). At 24h post-transfection, cells are lysed and polyadenylated mRNA is isolated with an mRNA CatcherTM CATCHERTM plate (Sequitur, Inc.), an mRNA isolation kit. Levels of HPTPbeta mRNA are determined by Real Time RT-PCR (Applied Biosystems) using ~~(TaqmanTM)~~ TAQMANTM (Applied Biosystems) gene expression assays and normalized to GAPDH mRNA. The PCR primers and probe used for HPTPbeta amplification hybridize upstream of the predicted sites of AS oligo annealing. Three AS oligos elicited potent inhibition of HPTPbeta mRNA showing an average 73% decrease in HPTPbeta mRNA levels as compared to chemistry matched oligo controls (Antisense oligos available from Sequitur, Inc.- part numbers S17924, S17929, and S17930)."

Please delete the paragraph beginning at page 28, line 17, and insert the following paragraph therefore:

"Transfection in HUVEC's is performed with the above-mentioned antisense oligos at a 200nM final concentration using ~~Lipofectin~~ LIPOFECTINTM (Invitrogen), a reagent for transfection of endothelial cells and transfection of DNA or RNA into cell lines, in ~~OptiMEM~~ OPTIMEMTM - I (Gibco), a modification of Eagles Minimum Essential Medium, buffered with HEPES and sodium bicarbonate (2.4 g/L) and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, and growth factors, according to the manufacturers instructions. After a 2-hour transfection, the cells are returned to normal growth media (EGM, Clonetics) and after 48 hours, assayed for VEGFR2 and Tie-2 receptor activity as described in example 4. Uptake efficiency is monitored in every transfection using a fluorescently labeled oligonucleotide and determined to be >90% in every experiment."